

The demand must be filed directly with the competent International Preliminary Examining Authority or two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:

IPEA/ US

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:

The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examining Authority use only

Identification of IPEA		Date of receipt of DEMAND	
Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference	
		19957-159-2	
International application No.	International filing date (day/month/year)	(Earliest) Priority date (day/month/year)	
PCT/US03/23155	23 July 2003 (23.07.03)	23 July 2002 (23.07.02)	
Title of invention			
SYNTHESIS OF GLYCOPROTEINS USING BACTERIAL GLYCOSYLTRANSFERASES			
Box No. II APPLICANT(S)			
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)		Telephone No.:	
NEOSE TECHNOLOGIES, INC.		215.441.5890	
102 Witmer Road		Facsimile No.:	
Horsham, PA 19044		215.441.5896	
United States of America		Teleprinter No.:	
		Applicant's registration No. with the Office	
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US		US	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)			
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US		US	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)			
BEZILA, Daniel, James			
715 Red Lion Road, 2nd Floor			
Philadelphia, PA 19115			
United States of America			
State (that is, country) of nationality:		State (that is, country) of residence:	
US		US	
<input type="checkbox"/> Further applicants are indicated on a continuation sheet.			

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is ☒ agent ☐ common representative
 and ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.
☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.
☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation.
 The address must include postal code and name of country.)*

BASTIAN, Kevin, L.
 TOWNSEND AND TOWNSEND AND CREW LLP
 Two Embarcadero Center, Eighth Floor
 San Francisco, California 94111-3834
 United States of America

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415-576-0300

Teleprinter No.:

Agent's registration No. with the Office

34,774

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION**Statement concerning amendments:***

1. The applicant wishes the international preliminary examination to start on the basis of:

☒ the international application as originally filed

the description

☐

as originally filed

☒

as amended under Article 34

the claims

☒

as originally filed

☐

as amended under Article 19 (together with any accompanying statement)

☐

as amended under Article 34

the drawings

☒

as originally filed

☐

as amended under Article 34

2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of applicable time limit under Rule 69.1(d).

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: ENGLISH

☒ which is the language in which the international application was filed.

☐ which is the language of a translation furnished for the purposes of international search.

☐ which is the language of publication of the international application.

☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The filing of this demand constitutes the election of all Contracting States which are designated and are bound by Chapter II of the PCT.

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

- | | | | |
|--|---|---|--------|
| 1. translation of international application | : | | sheets |
| 2. amendments under Article 34 | : | 7 | sheets |
| 3. copy (or, where required, translation) of amendments under Article 19 | : | | sheets |
| 4. copy (or, where required, translation) of statement under Article 19 | : | | sheets |
| 5. letter | : | 1 | sheets |
| 6. other (<i>specify</i>) | : | | sheets |

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received not received

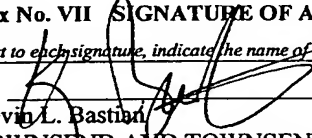
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<input type="checkbox"/>	<input type="checkbox"/>

The demand is also accompanied by the item(s) marked below:

- | | |
|--|---|
| 1. <input checked="" type="checkbox"/> fee calculation sheet | 5. <input type="checkbox"/> statement explaining lack of signature |
| 2. <input type="checkbox"/> original separate power of attorney | 6. <input type="checkbox"/> sequence listing in computer readable form |
| 3. <input type="checkbox"/> original general power of attorney | 7. <input type="checkbox"/> tables in computer readable form related to a sequence listing |
| 4. <input type="checkbox"/> copy of general power of attorney; reference number, if any: ; | 8. <input checked="" type="checkbox"/> other (<i>specify</i>): Transmittal Letter; Art. 34 Amendment w/7 substitute specification pages (w/redlined copies); Postcard |

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

X 
 Kevin L. Bastian
 TOWNSEND AND TOWNSEND AND CREW LLP
 USPTO Reg. No.: 34,774
 Applicants' Agent

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:	
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):	
3. <input type="checkbox"/> The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. <input type="checkbox"/> The applicant has been informed accordingly.	6. <input type="checkbox"/> The date of receipt of the demand is AFTER the expiration of the time limit under Rule 54bis.1(a) and item 7 or 8, below, does not apply
4. <input type="checkbox"/> The date of receipt of the demand is WITHIN the time limit of 19 months from the priority date as extended by virtue of Rule 80.5.	7. <input type="checkbox"/> The date of receipt of the demand is WITHIN the time limit under Rule 54bis.1(a) as extended by virtue of Rule 80.5.
5. <input type="checkbox"/> Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.	8. <input type="checkbox"/> Although the date of receipt of the demand is after the expiration of the time limit under Rule 54bis.1(a), the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

PCT

FEE CALCULATION SHEET

Annex to the Demand

For International Preliminary Examining Authority use only

International application No. PCT/US03/23155		
Applicant's or agent's file reference 19957-159-2	Date stamp of the IPEA	
Applicant NEOSE TECHNOLOGIES, INC.		
CALCULATION OF PRESCRIBED FEES		
1. Preliminary examination fee	<div style="border: 1px solid black; padding: 2px;">\$600.00</div>	<div style="border: 1px solid black; padding: 2px; width: 20px;">P</div>
2. Handling fee (<i>Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.</i>)	<div style="border: 1px solid black; padding: 2px;">\$148.00</div>	<div style="border: 1px solid black; padding: 2px; width: 20px;">H</div>
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box	<div style="border: 1px solid black; padding: 2px; margin-bottom: 2px;">\$748.00</div> <div style="border: 1px solid black; padding: 2px;">TOTAL</div>	
MODE OF PAYMENT		
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <input checked="" type="checkbox"/> authorization to charge deposit account with the IPEA (see below) </div> <div style="width: 45%;"> <input type="checkbox"/> cash </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div style="width: 45%;"> <input type="checkbox"/> cheque </div> <div style="width: 45%;"> <input type="checkbox"/> revenue stamps </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div style="width: 45%;"> <input type="checkbox"/> postal money order </div> <div style="width: 45%;"> <input type="checkbox"/> coupons </div> </div> <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div style="width: 45%;"> <input type="checkbox"/> bank draft </div> <div style="width: 45%;"> <input type="checkbox"/> other (specify): </div> </div>		
<div style="display: flex; justify-content: space-between;"> <div style="width: 55%;"> AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT <i>(This mode of payment may not be available at all IPEAs)</i> <div style="margin-top: 10px;"> <input checked="" type="checkbox"/> Authorization to charge the total fees indicated above. </div> <div style="margin-top: 10px;"> <input checked="" type="checkbox"/> <i>deposit accounts of the IPEA so permit</i> Authorization to charge any deficiency or credit any overpayment in the total fees indicated above. </div> </div> <div style="width: 40%;"> IPEA/ <u>US</u> Deposit Account No.: <u>20-1430</u> Date: <u>23 February 2004</u> Name: <u>Kevin L. Basman</u> Signature: </div> </div>		

TOWNSEND
and
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10/521138
DT12 PGT/PTO 12 JAN 2005

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23 February 2004

VIA EXPRESS MAIL, WITH RETURN POSTCARD ENCLOSED

PCT International Application Processing Div.
USPTO International Division
Assistant Commissioner for Patents
Mail Stop PCT
PO Box 1450
Alexandria, VA 22313-1450

Re: International Application No. PCT/US03/23155
Title: SYNTHESIS OF GLYCOPROTEINS USING BACTERIAL
GYCOSYLTRANSFERASES
Applicant: NEOSE TECHNOLOGIES, INC.
International Filing Date: 23 July 2003
Express Mail Label No.: EV 332 020 821 US
Date of Mailing: 23 February 2004
Our File No.: 19957-159-2

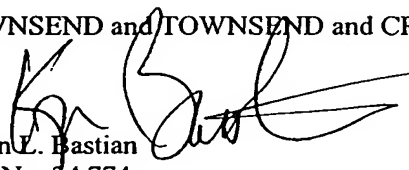
Dear Examiner:

Enclosed is the Chapter II Demand for the above-referenced application. Also enclosed are substitute pages 5, 5a, 6, 6a, 13, 34 and 40 of the Specification submitted as an Article 34 Amendment (redlined version of pages are enclosed). The changes to the pages were insertion of SEQ ID: NOs. These changes do not go beyond the disclosure of the application as filed.

Thank you for your attention to this matter.

Respectfully submitted,

TOWNSEND and TOWNSEND and CREW LLP


Kevin L. Bastian
Reg. No. 34,774

BLK/ljb

Enclosures:

Chapter II Demand w/Fee Calculation Sheet
Amendment under Art. 34
Substitute pages 5, 5a, 6, 6a, 13, 34 and 40 (7 pages)
Redlined version of substitute pages (5 pgs)
Transmittal Letter and Postcard

60139128 v1

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Figure 1 provides the nucleic acid (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequences of fucosyltransferase from *H. pylori* strain 1182B.

[0019] Figure 2 provides the nucleic acid (SEQ ID NO:3) and amino acid (SEQ ID NO:4) sequences of fucosyltransferase from *H. pylori* strain 1111A.

[0020] Figure 3 provides the nucleic acid (SEQ ID NO:5) and amino acid (SEQ ID NO:6) sequences of fucosyltransferase from *H. pylori* strain 1218B.

[0021] Figure 4 provides the nucleic acid (SEQ ID NO:7) and amino acid (SEQ ID NO:8) sequences of fucosyltransferase from *H. pylori* strain 19C2B.

[0022] Figure 5 provides the nucleic acid (SEQ ID NO:9) and amino acid (SEQ ID NO:10) sequences of fucosyltransferase from *H. pylori* strain 915A.

[0023] Figure 6 provides the nucleic acid (SEQ ID NO:11) and amino acid (SEQ ID NO:12) sequences of fucosyltransferase from *H. pylori* strain 26695A.

[0024] Figure 7 provides the nucleic acid (SEQ ID NO:13) and amino acid (SEQ ID NO:14) sequences of fucosyltransferase from *H. pylori* strain 19C2A.

[0025] Figure 8 provides an alignment between 1182 futB amino acid sequence (SEQ ID NO:15) and a consensus sequence from the glycosyltransferase family 10 (SEQ ID NO:16), *i.e.*, the fucosyltransferase family. Amino acids 23 through 305 of 1182 futB are shown in the top line and represent the most conserved region of the protein, *i.e.* the fucosyltransferase catalytic domain.

[0026] Figure 9 provides an alignment between 1111 futA amino acid sequence (SEQ ID NO:17) and a consensus sequence from the glycosyltransferase family 10 (SEQ ID NO:18), *i.e.*, the fucosyltransferase family. Amino acids 27 through 417 of 1182 futB are shown in the top line and represent the most conserved region of the protein, *i.e.* the fucosyltransferase catalytic domain.

[0027] Figure 10 provides an alignment between 1218 futB amino acid sequence (SEQ ID NO:19) and a consensus sequence from the glycosyltransferase family 10 (SEQ ID NO:20), *i.e.*, the fucosyltransferase family. Amino acids 23 through 399 of 1182 futB are shown in the top line and represent the most conserved region of the protein, *i.e.* the fucosyltransferase catalytic domain.

[0028] Figure 11 provides an alignment between 19C2 futB amino acid sequence (SEQ ID NO:21) and a consensus sequence from the glycosyltransferase family 10 (SEQ ID NO:22), *i.e.*, the fucosyltransferase

family. Amino acids 23 through 377 of 1182 futB are shown in the top line and represent the most conserved region of the disclosed protein, *i.e.* the fucosyltransferase catalytic domain.

[0029] Figure 12 provides an alignment between amino acid sequence of *H. pylori* strains 1182 FutB (SEQ ID NO:25), 1111 FutA (SEQ ID NO:23), 1218 FutB (SEQ ID NO:26),
5 19C2 FutB (SEQ ID NO:27), 915FutA (SEQ ID NO:10), 19C2 FutA (SEQ ID NO:14), and 26695 FutA (SEQ ID NO:24). The bottom sequence is a consensus sequence (SEQ ID NOS:28-37).

[0030] Figure 13 provides an alignment between nucleic acid sequence of *H. pylori* strains 1182 FutB (SEQ ID NO:1), 1111 FutA (SEQ ID NO:3), 1218 FutB (SEQ ID NO:5), 19C2
10 FutB (SEQ ID NO:7), 915FutA (SEQ ID NO:38), 19C2 FutA (SEQ ID NO:13), and 26695 FutA (SEQ ID NO:11). The bottom sequence is a consensus sequence (SEQ ID NOS:39-74).

[0031] Figure 14 provides oligosaccharide structures of Lacto-N-neo-Tetraose (LNnT), a substrate of the *H. pylori* fucosyltransferases and Lacto-N-Fucopentaose III (LNFPIII or LNFIII), a product of the *H. pylori* fucosyltransferases.


15 [0032] Figure 15 provides the results of analysis of acceptor specificity for the *H. pylori* fucosyltransferases.

[0033] Figure 16 provides the yield of LNFIII synthesis using the *H. pylori* fucosyltransferases. Two ion exchange resins were tested: MR3 NH₄HCO₃ and Dowex1/Dowex50 resin.

20 [0034] Figure 17 demonstrates the use of FutB α -1,3/4-fucosyltransferase from *H. pylori* strain 1182 to transfer fucose to the glycoprotein asialyltransferin. The upper panel shows GC/MS analysis of sialylated transferrin. The lower panel shows GC/MS analysis of sialylated transferrin that has been enzymatically asialylated and then fucosylated using *H. pylori* strain 1182 FutB α -1,3/4-fucosyltransferase. Key to sugar structures: filled squares-
25 GlcNAc; open circles-mannose; filled diamonds-galactose; triangles-fucose; stars-sialic acid.

DEFINITIONS

[0035] Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which
30 this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry and nucleic acid chemistry



and hybridization described below are those well known and commonly employed in the art. Standard techniques are used for nucleic acid and peptide synthesis. Generally, enzymatic reactions and purification steps are performed according to the manufacturer's specifications.

protein sequence that is specifically recognized by an antibody. Epitope tags are generally incorporated into fusion proteins to enable the use of a readily available antibody to unambiguously detect or isolate the fusion protein. A “FLAG tag” is a commonly used epitope tag, specifically recognized by a monoclonal anti-FLAG antibody, consisting of the sequence AspTyrLysAspAspAspLys (SEQ ID NO:75) or a substantially identical variant thereof. Other suitable tags are known to those of skill in the art, and include, for example, an affinity tag such as a hexahistidine (SEQ ID NO:76) peptide, which will bind to metal ions such as nickel or cobalt ions. Purification tags also include maltose binding domains and starch binding domains. Purification of maltose binding domain proteins is known to those of skill in the art. Starch binding domains are described in WO 99/15636, herein incorporated by reference. Affinity purification of a fusion protein comprising a starch binding domain using a betacylodextrin (BCD)-derivatized resin is described in USSN 60/468,374, filed May 5, 2003, herein incorporated by reference in its entirety.

[0055] The term “functional domain” with reference to glycosyltransferases, refers to a domain of the glycosyltransferase that confers or modulates an activity of the enzyme, *e.g.*, acceptor substrate specificity, catalytic activity, binding affinity, or other biological or biochemical activity. Examples of functional domains of glycosyltransferases include, but are not limited to, the catalytic domain.

[0056] The terms “expression level” or “level of expression” with reference to a protein refers to the amount of a protein produced by a cell. The amount of protein produced by a cell can be measured by the assays and activity units described herein or known to one skilled in the art. One skilled in the art would know how to measure and describe the amount of protein produced by a cell using a variety of assays and units, respectively. Thus, the quantitation and quantitative description of the level of expression of a protein, *e.g.*, an *H. pylori* fucosyltransferase, can be assayed measuring the enzymatic activity or the units used to describe the activity, or the amount of protein. The amount of protein produced by a cell can be determined by standard known assays, for example, the protein assay by Bradford (1976), the bicinchoninic acid protein assay kit from Pierce (Rockford, Illinois), or as described in U.S. Patent No. 5,641,668.

[0057] The term “enzymatic activity” refers to an activity of an enzyme and may be measured by the assays and units described herein or known to one skilled in the art.

junction of the two moieties results in production of a protein having an intact authentic N-terminal residue.

D. Purification of α -1,3/4-fucosyltransferase proteins

[0117] The *H. pylori* fucosyltransferase proteins of the present invention can be expressed as intracellular proteins or as proteins that are secreted from the cell, and can be used in this form, in the methods of the present invention. For example, a crude cellular extract containing the expressed intracellular or secreted *H. pylori* fucosyltransferase protein can be used in the methods of the present invention.

[0118] Alternatively, the *H. pylori* fucosyltransferase proteins can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (*see, generally, R. Scopes, Protein Purification*, Springer-Verlag, N.Y. (1982), Deutscher, *Methods in Enzymology Vol. 182: Guide to Protein Purification*, Academic Press, Inc. N.Y. (1990)). Substantially pure compositions of at least about 70 to 90% homogeneity are preferred, and 98 to 99% or more homogeneity are most preferred. The purified proteins may also be used, *e.g.*, as immunogens for antibody production.

[0119] To facilitate purification of the *H. pylori* α -1,3/4-fucosyltransferase proteins of the invention, the nucleic acids that encode the fusion proteins can also include a coding sequence for an epitope or "tag" for which an affinity binding reagent is available, *i.e.* a purification tag. Examples of suitable epitopes include the myc and V-5 reporter genes; expression vectors useful for recombinant production of fusion proteins having these epitopes are commercially available (*e.g.*, Invitrogen (Carlsbad CA) vectors pcDNA3.1/Myc-His and pcDNA3.1/V5-His are suitable for expression in mammalian cells). Additional expression vectors suitable for attaching a tag to the *H. pylori* α -1,3/4-fucosyltransferase proteins of the invention, and corresponding detection systems are known to those of skill in the art, and several are commercially available (*e.g.*, FLAG" (Kodak, Rochester NY). Another example of a suitable tag is a polyhistidine sequence, which is capable of binding to metal chelate affinity ligands. Typically, six adjacent histidines (SEQ ID NO:76) are used, although one can use more or less than six. Suitable metal chelate affinity ligands that can serve as the binding moiety for a polyhistidine tag include nitrilo-tri-acetic acid (NTA) (Hochuli, E. (1990) "Purification of recombinant proteins with metal chelating adsorbents" In Genetic Engineering: Principles

for affinity-based immobilization. For example, antibodies that specifically bind to a glycoprotein are suitable. Also, where the glycoprotein of interest is itself an antibody or contains a fragment thereof, one can use protein A or G as the affinity resin. Dyes and other molecules that specifically bind to a glycoprotein or glycolipid of interest are also suitable.

5 [0135] The recombinant fusion protein of the invention can be constructed and expressed as a fusion protein with a molecular “tag” at one end, which facilitates purification of the protein, *i.e.*, a purification tag. Such tags can also be used for immobilization of a protein of interest during the glycosylation reaction. Suitable tags include “epitope tags,” which are a protein sequence that is specifically recognized by an antibody. Epitope tags are generally
10 incorporated into fusion proteins to enable the use of a readily available antibody to unambiguously detect or isolate the fusion protein. A “FLAG tag” is a commonly used epitope tag, specifically recognized by a monoclonal anti-FLAG antibody, consisting of the sequence AspTyrLysAspAspAspAspLys (SEQ ID NO:75) or a substantially identical variant thereof. A myc tag is another commonly used epitope tag. Other suitable tags are known to
15 those of skill in the art, and include, for example, an affinity tag such as a hexahistidine (SEQ ID NO:76) peptide, which will bind to metal ions such as nickel or cobalt ions. Purification tags also include maltose binding domains and starch binding domains. Purification of maltose binding domain proteins is known to those of skill in the art. Starch binding domains are described in WO 99/15636, herein incorporated by reference. Affinity purification of a
20 fusion protein comprising a starch binding domain using a betacylodextrin (BCD)-derivatized resin is described in USSN 60/468,374, filed May 5, 2003, herein incorporated by reference in its entirety.

[0136] Preferably, when the glycoprotein is a truncated version of the full-length glycoprotein, it preferably includes the biologically active subsequence of the full-length
25 glycoprotein. Exemplary biologically active subsequences include, but are not limited to, enzyme active sites, receptor binding sites, ligand binding sites, complementarity determining regions of antibodies, and antigenic regions of antigens.

[0137] In some embodiments, the *H. pylori* fucosyltransferase proteins and methods of the present invention are used to enzymatically synthesize a glycoprotein or glycolipid that has a
30 substantially uniform glycosylation pattern. The glycoproteins and glycolipids include a saccharide or oligosaccharide that is attached to a protein, glycoprotein, lipid, or glycolipid for which a glycoform alteration is desired. The saccharide or oligosaccharide includes a

BRIEF DESCRIPTION OF THE DRAWINGS

- [0018] Figure 1 provides the nucleic acid^(SEQ ID No:1) and amino acid^(SEQ ID No:2) sequences of fucosyltransferase from *H. pylori* strain 1182B.
- 5 [0019] Figure 2 provides the nucleic acid^(SEQ ID No:3) and amino acid^(SEQ ID No:4) sequences of fucosyltransferase from *H. pylori* strain 1111A.
- [0020] Figure 3 provides the nucleic acid^(SEQ ID No:5) and amino acid^(SEQ ID No:6) sequences of fucosyltransferase from *H. pylori* strain 1218B.
- 10 [0021] Figure 4 provides the nucleic acid^(SEQ ID No:7) and amino acid^(SEQ ID No:8) sequences of fucosyltransferase from *H. pylori* strain 19C2B.
- [0022] Figure 5 provides the nucleic acid^(SEQ ID No:9) and amino acid^(SEQ ID No:10) sequences of fucosyltransferase from *H. pylori* strain 915A.
- [0023] Figure 6 provides the nucleic acid^(SEQ ID No:11) and amino acid^(SEQ ID No:12) sequences of fucosyltransferase from *H. pylori* strain 26695A.
- 15 [0024] Figure 7 provides the nucleic acid^(SEQ ID No:13) and amino acid^(SEQ ID No:14) sequences of fucosyltransferase from *H. pylori* strain 19C2A.
- [0025] Figure 8 provides an alignment between 1182 futB amino acid sequence^(SEQ ID No:15) and a consensus sequence from the glycosyltransferase family 10^(SEQ ID No:16), *i.e.*, the fucosyltransferase family. Amino acids 23 through 305 of 1182 futB are shown in the top line and represent the
- 20 most conserved region of the protein, *i.e.* the fucosyltransferase catalytic domain.
- [0026] Figure 9 provides an alignment between 1111 futA amino acid sequence^(SEQ ID No:17) and a consensus sequence from the glycosyltransferase family 10^(SEQ ID No:18), *i.e.*, the fucosyltransferase family. Amino acids 27 through 417 of 1182 futB are shown in the top line and represent the most conserved region of the protein, *i.e.* the fucosyltransferase catalytic domain.
- 25 [0027] Figure 10 provides an alignment between 1218 futB amino acid sequence^(SEQ ID No:19) and a consensus sequence from the glycosyltransferase family 10^(SEQ ID No:20), *i.e.*, the fucosyltransferase family. Amino acids 23 through 399 of 1182 futB are shown in the top line and represent the most conserved region of the protein, *i.e.* the fucosyltransferase catalytic domain.
- [0028] Figure 11 provides an alignment between 19C2 futB amino acid sequence^(SEQ ID No:21) and a consensus sequence from the glycosyltransferase family 10^(SEQ ID No:22), *i.e.*, the fucosyltransferase
- 30

family. Amino acids 23 through 377 of 1182 futB are shown in the top line and represent the most conserved region of the disclosed protein, i.e. the fucosyltransferase catalytic domain.

[0029] Figure 12 provides an alignment between amino acid sequence of *H. pylori* strains
(SEQ ID NO:1) (SEQ ID NO:23) (SEQ ID NO:26) (SEQ ID NO:27) (SEQ ID NO:10) (SEQ ID NO:14) (SEQ ID NO:24)
1182 FutB_A, 1111 FutA_A, 1218 FutB_A, 19C2 FutB_A, 915FutA_A, 19C2 FutA_A, and 26695 FutA_A. The
5 bottom sequence is a consensus sequence_A (SEQ ID NO:28-37)

[0030] Figure 13 provides an alignment between nucleic acid sequence of *H. pylori* strains
(SEQ ID NO:1) (SEQ ID NO:3) (SEQ ID NO:5) (SEQ ID NO:7) (SEQ ID NO:38) (SEQ ID NO:13) (SEQ ID NO:11)
1182 FutB_A, 1111 FutA_A, 1218 FutB_A, 19C2 FutB_A, 915FutA_A, 19C2 FutA_A, and 26695 FutA_A. The
bottom sequence is a consensus sequence_A (SEQ ID NO:39-74)

[0031] Figure 14 provides oligosaccharide structures of Lacto-N-neo-Tetraose (LNnT), a
10 substrate of the *H. pylori* fucosyltransferases and Lacto-N-Fucopentaose III (LNFPIII or
LNFIII), a product of the *H. pylori* fucosyltransferases.

[0032] Figure 15 provides the results of analysis of acceptor specificity for the *H. pylori*
fucosyltransferases.

[0033] Figure 16 provides the yield of LNFIII synthesis using the *H. pylori*
15 fucosyltransferases. Two ion exchange resins were tested: MR3 NH₄HCO₃ and
Dowex1/Dowex50 resin.

[0034] Figure 17 demonstrates the use of FutB α-1,3/4-fucosyltransferase from *H. pylori*
strain 1182 to transfer fucose to the glycoprotein asialyltransferin. The upper panel shows
GC/MS analysis of sialylated transferrin. The lower panel shows GC/MS analysis of
20 sialylated transferrin that has been enzymatically asialylated and then fucosylated using *H.*
pylori strain 1182 FutB α-1,3/4-fucosyltransferase. Key to sugar structures: filled squares-
GlcNAc; open circles-mannose; filled diamonds-galactose; triangles-fucose; stars-sialic acid.

DEFINITIONS

25 [0035] Unless defined otherwise, all technical and scientific terms used herein generally
have the same meaning as commonly understood by one of ordinary skill in the art to which
this invention belongs. Generally, the nomenclature used herein and the laboratory
procedures in cell culture, molecular genetics, organic chemistry and nucleic acid chemistry
and hybridization described below are those well known and commonly employed in the art.
30 Standard techniques are used for nucleic acid and peptide synthesis. Generally, enzymatic
reactions and purification steps are performed according to the manufacturer's specifications.

protein sequence that is specifically recognized by an antibody. Epitope tags are generally incorporated into fusion proteins to enable the use of a readily available antibody to unambiguously detect or isolate the fusion protein. A "FLAG tag" is a commonly used epitope tag, specifically recognized by a monoclonal anti-FLAG antibody, consisting of the sequence AspTyrLysAspAspAsp^(SEQ ID No: 75)AspLys_A or a substantially identical variant thereof. Other suitable tags are known to those of skill in the art, and include, for example, an affinity tag such as a hexahistidine^(SEQ ID No: 76)peptide, which will bind to metal ions such as nickel or cobalt ions. Purification tags also include maltose binding domains and starch binding domains. Purification of maltose binding domain proteins is known to those of skill in the art. Starch binding domains are described in WO 99/15636, herein incorporated by reference. Affinity purification of a fusion protein comprising a starch binding domain using a betacylodextrin (BCD)-derivatized resin is described in USSN 60/468,374, filed May 5, 2003, herein incorporated by reference in its entirety.

[0055] The term "functional domain" with reference to glycosyltransferases, refers to a domain of the glycosyltransferase that confers or modulates an activity of the enzyme, *e.g.*, acceptor substrate specificity, catalytic activity, binding affinity, or other biological or biochemical activity. Examples of functional domains of glycosyltransferases include, but are not limited to, the catalytic domain.

[0056] The terms "expression level" or "level of expression" with reference to a protein refers to the amount of a protein produced by a cell. The amount of protein produced by a cell can be measured by the assays and activity units described herein or known to one skilled in the art. One skilled in the art would know how to measure and describe the amount of protein produced by a cell using a variety of assays and units, respectively. Thus, the quantitation and quantitative description of the level of expression of a protein, *e.g.*, an *H. pylori* fucosyltransferase, can be assayed measuring the enzymatic activity or the units used to describe the activity, or the amount of protein. The amount of protein produced by a cell can be determined by standard known assays, for example, the protein assay by Bradford (1976), the bicinchoninic acid protein assay kit from Pierce (Rockford, Illinois), or as described in U.S. Patent No. 5,641,668.

[0057] The term "enzymatic activity" refers to an activity of an enzyme and may be measured by the assays and units described herein or known to one skilled in the art.

junction of the two moieties results in production of a protein having an intact authentic N-terminal residue.

D. Purification of α -1,3/4-fucosyltransferase proteins

[0117] The *H. pylori* fucosyltransferase proteins of the present invention can be expressed as intracellular proteins or as proteins that are secreted from the cell, and can be used in this form, in the methods of the present invention. For example, a crude cellular extract containing the expressed intracellular or secreted *H. pylori* fucosyltransferase protein can be used in the methods of the present invention.

[0118] Alternatively, the *H. pylori* fucosyltransferase proteins can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (*see, generally, R. Scopes, Protein Purification*/Springer-Verlag, N.Y. (1982), Deutscher, *Methods in Enzymology Vol. 182: Guide to Protein Purification*., Academic Press, Inc. N.Y. (1990)). Substantially pure compositions of at least about 70 to 90% homogeneity are preferred, and 98 to 99% or more homogeneity are most preferred. The purified proteins may also be used, *e.g.*, as immunogens for antibody production.

[0119] To facilitate purification of the *H. pylori* α -1,3/4-fucosyltransferase proteins of the invention, the nucleic acids that encode the fusion proteins can also include a coding sequence for an epitope or "tag" for which an affinity binding reagent is available, *i.e.* a purification tag. Examples of suitable epitopes include the myc and V-5 reporter genes; expression vectors useful for recombinant production of fusion proteins having these epitopes are commercially available (*e.g.*, Invitrogen (Carlsbad CA) vectors pcDNA3.1/Myc-His and pcDNA3.1/V5-His are suitable for expression in mammalian cells). Additional expression vectors suitable for attaching a tag to the *H. pylori* α -1,3/4-fucosyltransferase proteins of the invention, and corresponding detection systems are known to those of skill in the art, and several are commercially available (*e.g.*, FLAG" (Kodak, Rochester NY). Another example of a suitable tag is a polyhistidine sequence, which is capable of binding to metal chelate affinity ligands. Typically, six adjacent histidines^(SEQ ID No:76) are used, although one can use more or less than six. Suitable metal chelate affinity ligands that can serve as the binding moiety for a polyhistidine tag include nitrilo-tri-acetic acid (NTA) (Hochuli, E. (1990) "Purification of recombinant proteins with metal chelating adsorbents" In Genetic Engineering: Principles

for affinity-based immobilization. For example, antibodies that specifically bind to a glycoprotein are suitable. Also, where the glycoprotein of interest is itself an antibody or contains a fragment thereof, one can use protein A or G as the affinity resin. Dyes and other molecules that specifically bind to a glycoprotein or glycolipid of interest are also suitable.

5 [0135] The recombinant fusion protein of the invention can be constructed and expressed as a fusion protein with a molecular "tag" at one end, which facilitates purification of the protein, *i.e.*, a purification tag. Such tags can also be used for immobilization of a protein of interest during the glycosylation reaction. Suitable tags include "epitope tags," which are a protein sequence that is specifically recognized by an antibody. Epitope tags are generally
10 incorporated into fusion proteins to enable the use of a readily available antibody to unambiguously detect or isolate the fusion protein. A "FLAG tag" is a commonly used epitope tag, specifically recognized by a monoclonal anti-FLAG antibody, consisting of the sequence AspTyrLysAspAspAsp^(SEQ ID No: 75)AspLys_A or a substantially identical variant thereof. A myc tag is another commonly used epitope tag. Other suitable tags are known to those of skill in
15 the art, and include, for example, an affinity tag such as a hexahistidine^(SEQ ID No: 76)peptide, which will bind to metal ions such as nickel or cobalt ions. Purification tags also include maltose binding domains and starch binding domains. Purification of maltose binding domain proteins is known to those of skill in the art. Starch binding domains are described in WO 99/15636, herein incorporated by reference. Affinity purification of a fusion protein
20 comprising a starch binding domain using a betacylodextrin (BCD)-derivatized resin is described in USSN 60/468,374, filed May 5, 2003, herein incorporated by reference in its entirety.

[0136] Preferably, when the glycoprotein is a truncated version of the full-length glycoprotein, it preferably includes the biologically active subsequence of the full-length
25 glycoprotein. Exemplary biologically active subsequences include, but are not limited to, enzyme active sites, receptor binding sites, ligand binding sites, complementarity determining regions of antibodies, and antigenic regions of antigens.

[0137] In some embodiments, the *H. pylori* fucosyltransferase proteins and methods of the present invention are used to enzymatically synthesize a glycoprotein or glycolipid that has a
30 substantially uniform glycosylation pattern. The glycoproteins and glycolipids include a saccharide or oligosaccharide that is attached to a protein, glycoprotein, lipid, or glycolipid for which a glycoform alteration is desired. The saccharide or oligosaccharide includes a

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VIII-4-1	<p>Declaration: Inventorship (only for the purposes of the designation of the United States of America) Declaration of Inventorship (Rules 4.17(iv) and 51bis.1(a)(iv)) for the purposes of the designation of the United States of America:</p>	<p>I hereby declare that I believe I am the original, first and sole (if only one inventor is listed below) or joint (if more than one inventor is listed below) inventor of the subject matter which is claimed and for which a patent is sought.</p> <p>This declaration is directed to the international application of which it forms a part (if filing declaration with application).</p> <p>I hereby declare that my residence, mailing address, and citizenship are as stated next to my name.</p> <p>I hereby state that I have reviewed and understand the contents of the above-identified international application, including the claims of said application. I have identified in the request of said application, in compliance with PCT Rule 4.10, any claim to foreign priority, and I have identified below, under the heading "Prior Applications," by application number, country or Member of the World Trade Organization, day, month and year of filing, any application for a patent or inventor's certificate filed in a country other than the United States of America, including any PCT international application designating at least one country other than the United States of America, having a filing date before that of the application on which foreign priority is claimed.</p>
VIII-4-1 -1	Prior applications:	